CLAIMS

1. A conjugate comprising at least one non-polypeptide moiety covalently attached to a protein C polypeptide that comprises an amino acid sequence which differs from that of a parent protein C polypeptide in at least one introduced and/or at least one removed amino acid residue comprising an attachment group for said non-polypeptide moiety.

2. The conjugate according to claim 1, wherein the parent protein C polypeptide has the amino acid sequence shown in SEQ ID NO:4 or is a variant thereof.

3. The conjugate according to claim 2, wherein the parent protein C polypeptide has the amino acid sequence shown in SEQ ID NO:4.

4. The conjugate according to claim 1 in its activated form.

5. The conjugate according to claim 1, wherein at least one attachment group for the non-polypeptide moiety has been introduced.

6. The conjugate according to claim 5, wherein at least one glycosylation site has been introduced.

7. The conjugate according to claim 6, wherein the glycosylation site is an *in vivo* N-glycosylation site.

8. The conjugate according to claim 7, wherein the glycosylation site has been introduced in a position which is occupied by an amino acid residue having at least 25% of its side chain exposed to the surface (as defined in Example 1 herein).

9. The conjugate according to claim 8, wherein the introduced glycosylation site is selected from the group consisting of D172N+K174S, D172N+K174T, D189N+K191S, D189N+K191T, S190N+K192S, S190N+K192T, K191N+K193S, K191N+K193T, K192N+L194S, K192N+L194T, K193N+A195S, K193N+A195T, D214N, D214N+S216T, E215N+K217S, E215N+K217T, S216N+K218S, S216N+K218T, K217N+L219S,

K217N+L219T, K218N+L220S, K218N+L220T, L220N+R222S, L220N+R222T, V243N+V245S, V243N+V245T, V245N+P247S, V245N+P247T, S250N, S250N+S252T, K251N, K251N+T253S, S252N, S252N+T254S, T253N+D255S, T253N+D255T, T254N+N256S, T254N+N256T, D255N+D257S, D255N+D257T, L296N, L296N+T298S, Y302N, Y302N+S304T, H303N, H303N+S305T, S304N+R306S, S304N+R306T, S305N+E307S, S305N+E307T, R306N+K308S, R306N+K308T, E307N+E309S, E307N+E309T, K308N+A310S, K308N+A310T, E309N+K311S, E309N+K311T, A310N+R312S, A310N+R312T, R312N+R314S, R312N+R314T, T315N+V317S, T315N+V317T, F316N+L318S, F316N+L318T, V334N, V334N+S336T, S336N+M338S, S336N+M338T, V339S, V339T, M338N, M338N+S340T, I348N+G350S, I348N+G350T, L349N+D351S, L349N+D351T, D351N+Q353S, D351N+Q353T, R352N+D354S, R352N+D354T, E357N+D359S, E357N+D359T, G383N+G385S, G383N+G385T, L386N+H388S, L386N+H388T, L387N+N389S, L387N+N389T, H388N+Y390S, and H388N+Y390T.

10. The conjugate according to claim 9, wherein the introduced glycosylation site is selected from the group consisting of D189N+K191S, D189N+K191T, S190N+K192S, S190N+K192T, K191N+K193S, K191N+K193T, D214N, D214N+S216T, K217N+L219S, K217N+L219T, K251N, K251N+T253S, S252N, S252N+T254S, T253N+D255S, T253N+D255T, Y302N, Y302N+S304T, T253N+D255S, T253N+D255T, S336N+M338S, S336N+M338T, V339S, V339T, M338N, M338N+S340T, G383N+G385S, G383N+G385T, L386N+H388S, and L386N+H388T.

11. The conjugate according to claim 10, wherein the introduced glycosylation site is selected from the group consisting of D189N+K191S, D189N+K191T, K191N+K193T, D214N, D214N+S216T, K251N, K251N+T253S, S252N, S252N+T254S, T253N+D255S, T253N+D255T, Y302N, Y302N+S304T, S305N+E307T, S305N+E307S, S336N+M338S, S336N+M338T, V339S, V339T, M338N, M338N+S340T, G383N+G385S, G383N+G385T, L386N+H388S and L386N+H388T.

12. The conjugate according to claim 11, wherein the introduced glycosylation site is selected from the group consisting of D189N+K191T, K191N+K193T, D214N, K251N, S252N,

T253N+D255T, Y302N, S305N+E307T, S336N+M338T, V339T, M338N, G383N+G385T, and L386N+H388T.

13. The conjugate according to claim 12, wherein the introduced glycosylation site is selected from the group consisting of D189N+K191T, K191N+K193T, D214N, T253N+D255T, S305N+E307T, S336N+M338T, M338N, G383N+G385T, and L386N+H388T.

14. The conjugate according to claim 13, wherein the introduced glycosylation site is selected from the group consisting of D189N+K191T, D214N, and L386N+H388T.

15. The conjugate according to claim 1, wherein the introduced and/or removed attachment group is selected from the group consisting of a lysine residue, a glutamic acid residue, an aspartic acid residue, a tyrosine residue, a serine residue, and a cysteine residue.

16. The conjugate according to claim 15, wherein the attachment group has been introduced in or removed from a position which is occupied by an amino acid residue having at least 25% of its side chain exposed to the surface (as defined in Example 1 herein).

17. The conjugate according to claim 16, wherein the attachment group is introduced in or removed from a position selected from the group consisting of D172, D189, S190, K191, K192, K193, D214, E215, S216, K217, K218, L220, V243, V245, S250, K251, S252, T253, T254, L296, Y302, H303, S304, S305, R306, E307, K308, E309, A310, R312, T315, F316, V334, S336, N337, M338, I348, L349, D351, R352, E357, G383, L386, L387, and H388.

18. The conjugate according to claim 17, wherein the attachment group is introduced in or removed from a position selected from the group consisting of D189, S190, K191, D214, K217, K251, S252, T253, Y302, S305, E307, S336, N337, M338, G383, and L386.

19. The conjugate according to claim 18, wherein the attachment group is introduced in a or removed from a position selected from the group consisting of D189, D214, K251, S252, T253, Y302, S305, S336, N337, M338, G383, and L386.

20. The conjugate according to claim 15, wherein the introduced attachment group is a cysteine residue.

21. The conjugate according to claim 15, wherein the non-polypeptide moiety is a polymer molecule.

22. The conjugate according to claim 21, wherein the non-polypeptide moiety is a linear or branched polyethylene glycol or a polyalkylene oxide.

23. The conjugate according to claim 5, which further comprises a non-polypeptide moiety, wherein the non-polypeptide moiety is a polymer molecule.

24. The conjugate according to claim 1, which, in its activated form, and when tested in the "APC Amidolytic Assay" described in Example 9 herein, has an activity of at least 10% of the wild-type human APC activity.

25. The conjugate according to claim 1, which, in its activated form, and when tested in the "APC Clotting Assay" described in Example 10, has an anticoagulant activity of at least 10% of the wild-type human APC anticoagulant activity.

26. The conjugate according to claim 1, which, in its activated form, has an increased resistance towards inactivation by alpha-1-antitrypsin as compared to human APC.

27. The conjugate according to claim 26, which, in its activated form has a residual activity of at least 20% when tested in the "Alpha-1-Antitrypsin Inactivation Assay" described in Example 11 herein using an inhibitor concentration of 16.6 μM.

28. The conjugate according to claim 1, which, in its activated form, has an increased resistance towards inactivation by human plasma.

29. The conjugate according to claim 28, which, in its activated form and when tested in the "Human Plasma Inactivation Assay I" described in Example 12 herein, has a residual activity of at least 20%.

30. The conjugate according to claim 28, wherein the ratio between the *in vitro* half-life of said conjugate in its activated form and the *in vitro* half-life of human APC is at least 1.25 when tested in the "Human Plasma Inactivation Assay II" described in Example 13 herein.

31. The conjugate according to claim 1, which, in its activated form, has an increased functional *in vivo* half-life or an increased serum half-life as compared to human APC.

32. The conjugate according to claim 31, wherein the ratio between the functional *in vivo* half-life or the serum half-life of said conjugate and the functional *in vivo* half-life or serum half-life of human APC is at least 1.25.

33. A variant of a parent protein C polypeptide, said variant comprising a substitution in a position sclected from the group consisting of D172, D189, S190, K191, K192, K193, D214, E215, S216, K217, K218, L220, V243, V245, S250, K251, S252, T253, T254, D255, L296, Y302, H303, S304, S305, R306, E307, K308, E309, A310, R312, T315, F316, V334, S336, N337, M338, I348, L349, D351, R352, E357, E382, G383, L386, L387, and H388, with the proviso that the substitution is not selected from the group consisting of T254S, T254A, T254H, T254K, T254R, T254N, T254D, T254E, T254G, T254Q, Y302S, Y302A, Y302T, Y302H, Y302K, Y302R, Y302N, Y302D, Y302E, Y302G, Y302Q, F316S, F316A, F316T, F316H, F316K, F316R, F316N, F316D, F316E, F316G, and F316Q.

34. The variant according to claim 33, wherein the parent protein C polypeptide has the amino acid sequence shown in SEQ ID NO:4.

35. The variant according to claim 33 in its activated form.

36. The variant according to claim 33, wherein said variant comprises at least one introduced *in vivo* N-glycosylation site selected from the group consisting of D172N+K174S, D172N+K174T, D189N+K191S, D189N+K191T, S190N+K192S, S190N+K192T, K191N+K193S, K191N+K193T, K192N+L194S, K192N+L194T, K193N+A195S, K193N+A195T, D214N, D214N+S216T, E215N+K217S, E215N+K217T, S216N+K218S, S216N+K218T, K217N+L219S, K217N+L219T, K218N+L220S, K218N+L220T,

L220N+R222S, L220N+R222T, V243N+V245S, V243N+V245T, V245N+P247S, V245N+P247T, S250N, S250N+S252T, K251N, K251N+T253S, S252N, S252N+T254S, T253N+D255S, T253N+D255T, T254N+N256S, T254N+N256T, D255N+D257S, D255N+D257T, L296N, L296N+T298S, Y302N+S304T, H303N, H303N+S305T, S304N+R306S, S304N+R306T, S305N+E307S, S305N+E307T, R306N+K308S, R306N+K308T, E307N+E309S, E307N+E309T, K308N+A310S, K308N+A310T, E309N+K311S, E309N+K311T, A310N+R312S, A310N+R312T, R312N+R314S, R312N+R314T, T315N+V317S, T315N+V317T, F316N+L318S, F316N+L318T, V334N, V334N+S336T, S336N+M338S, S336N+M338T, V339S, V339T, M338N, M338N+S340T, I348N+G350S, I348N+G350T, L349N+D351S, L349N+D351T, D351N+Q353S, D351N+Q353T, R352N+D354S, R352N+D354T, E357N+D359S, E357N+D359T, G383N+G385S, G383N+G385T, L386N+H388S, L386N+H388T, L387N+N389S, L387N+N389T, H388N+Y390S, and H388N+Y390T.

37. The variant according to claim 33, wherein said variant comprises a substitution selected from the group consisting of K251N, S252N, and Y302N.

38. The variant according to claim 33, wherein the variant in its activated form comprises at least one property selected from the group consisting of:

a) an activity of at least 10% of wild-type human APC activity when tested in the "APC Amidolytic Assay" described in Example 9;

b) an anticoagulant activity of at least 10% of wild-type human APC anticoagulant activity when tested in the "APC Clotting Assay" described in Example 10;

c) an increased resistance towards inactivation by alpha-1-antitrypsin as compared to human APC;

d) an increased resistance towards inactivation by human plasma as compared to human APC; and

e) an increased functional *in vivo* half-life or an increased serum half-life as compared to human APC.

39. The variant according to claim 38, wherein the variant in its activated form has a residual activity of at least 20% when tested in the "Alpha-1-Antitrypsin Inactivation Assay" described in Example 11 using an inhibitor concentration of 16.6 μM.

40. The variant according to claim 38, wherein the variant in its activated form has a residual activity of at least 20% when tested in the "Human Plasma Inactivation Assay I" described in Example 12.

41. The variant according to claim 38, wherein the ratio between the *in vitro* half-life of said variant in its activated form, and the *in vitro* half-life of human APC is at least 1.25 when tested in the "Human Plasma Inactivation Assay II" described in Example 13.

42. The variant according to claim 38, wherein the ratio between the functional *in vivo* half-life or the serum half-life of said variant and the functional *in vivo* half-life or serum half-life of human APC is at least 1.25.

43. A nucleotide sequence encoding the variant as defined in claim 33.

44. An expression vector comprising a nucleotide sequence as defined in claim 43.

45. A host cell comprising a nucleotide sequence as defined in claim 43 or an expression vector as defined in claim 44.

46. The host cell according to claim 45, which is selected from the group consisting of COS, CHO, BHK and HEK293 cells.

47. A pharmaceutical composition comprising a conjugate as defined in claim 1 and a pharmaceutically acceptable carrier or excipient.

48. A pharmaceutical composition comprising a variant as defined in claim 33 and a pharmaceutically acceptable carrier or excipient.

49. A method for treating or preventing a disease selected from the group consisting of stroke; myocardial infarction; after venous thrombosis; disseminated intravascular coagulation (DIC); sepsis; septic shock; emboli, such as pulmonary emboli; transplantation, such as bone marrow transplantation; burns; pregnancy; major surgery/traum and adult respiratory stress syndrome

(ARDS), the method comprising administering to a patient in need thereof an effective amount of

a) a conjugate as defined in claim 1,

b) a variant as defined in claim 33,

c) a pharmaceutical composition as defined in claim 47, or

d) a pharmaceutical composition as defined in claim 48.

50. The method according to claim 49 for treating or preventing septic shock.

51. A method for producing a conjugate as defined in claim 1, the method comprising culturing an appropriate host cell under conditions conducive for the expression of the polypeptide part of the conjugate, and recovering the polypeptide, wherein

a) the polypeptide comprises at least one N- or O-glycosylation site and the host cell is an eukaryotic host cell capable of *in vivo* glycosylation, and/or

b) the polypeptide is subjected to conjugation to a non-polypeptide moiety *in vitro*.

52. A method of increasing the functional *in vivo* half-life or the serum half-life of a parent protein C polypeptide, which method comprises:

a1) introducing an amino acid residue constituting an attachment group for a non-polypeptide moiety into a position of a parent protein C polypeptide comprising an amino acid residue having at least 25% of its side chain exposed to the surface (as defined in Example 1 herein) which does not contain such attachment group, and/or

a2) removing an amino acid residue constituting such attachment group, and

b) subjecting the resulting modified polypeptide to conjugation with the non-polypeptide moiety which has the amino acid residue having been introduced and/or removed as an the attachment group.